

Title: Inhibition of glycogen synthase kinase-3 by BTA-EG₄ reduces tau abnormalities in an organotypic brain slice culture model of Alzheimer's disease

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Supplementary Materials and Methods

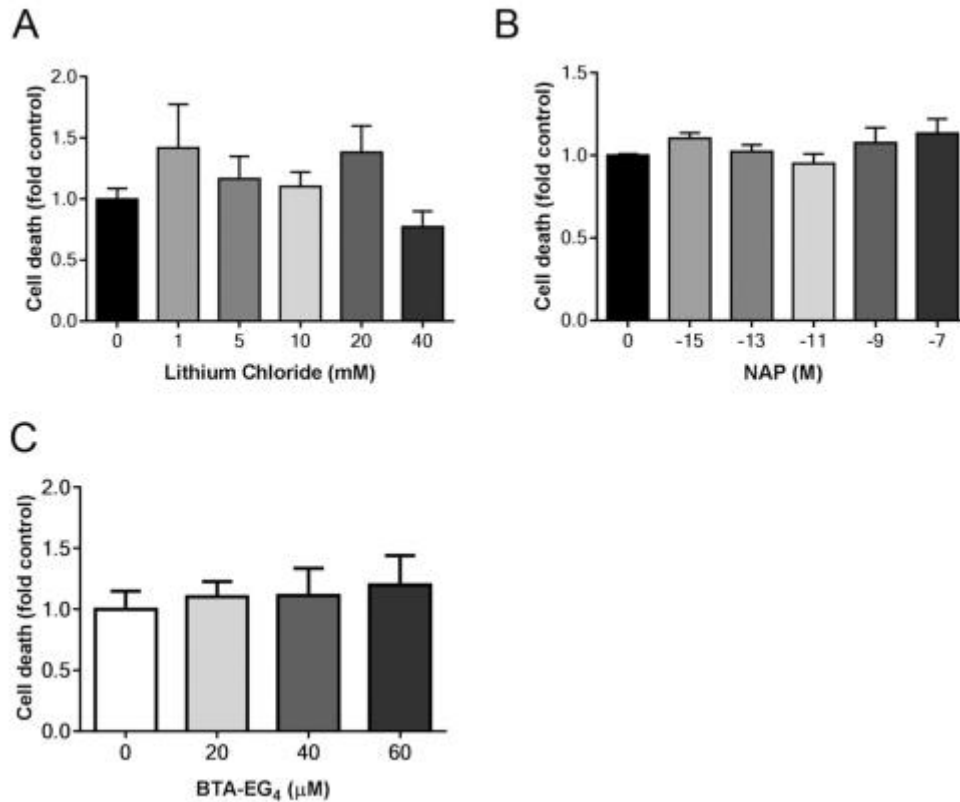
Primary cortical cell culture and treatments:

Primary cortical neuronal cultures were prepared from embryonic day 18 (E18) rat embryos as described previously (Atherton et al., 2014), plated onto glass coverslips in 12 well plates (125,000 cells/well) and maintained in culture for 7-10DIV dependent on treatment paradigm. For LiCl treatments, 10DIV primary cortical neurons were treated for 4 h with 1, 5, 10, 20, 40 mM LiCl before assessment of cell viability. 20 mM NaCl was used as vehicle. For NAPVSIPQ treatments, 7 DIV primary cortical neurons were treated for 24 h with 1 x 10⁻¹⁵, 10⁻¹³, 10⁻¹¹, 10⁻⁹, 10⁻⁷ M NAPVSIPQ or vehicle (ultrapure H₂O) before assessing cell viability. For BTA-EG₄ treatments, 7 DIV primary cortical neurons were treated for 24 h with 20, 40, 60 μM BTA-EG₄ or vehicle (DMSO) before assessing cell viability. N represents 1 well of a 12-well plate treated with drug or control, n=9 for all.

Cell death assays:

After treatment, culture medium was removed and neurons washed in pre-warmed PBS. Cell death was confirmed using a live/dead fixable cell stain (Invitrogen, Paisley, UK), performed according to the manufacturer's instructions. Cell death was quantified as a proportion of total cell death (cell dye uptake).

Supp. Fig. 1



Supplementary Figure 1: LiCl, NAP, and BTA-EG₄ effects on cell viability. Bar charts show levels of cell death, measured by incorporation of dead cell dye, following (A) treatment of 10 DIV primary cortical cultures with 1-40mM LiCl or control (20mM NaCl) for 4h, (B) treatment of 7 DIV primary cortical cultures with 1×10^{-15} – 1×10^{-7} M NAP treatment or control (H₂O) for 24h, (C) treatment of 7 DIV primary cortical cultures for 24h with control (DMSO, 0μM), 20, 40 or 60 μM BTA-EG₄. Data is shown as fold change from control. Data is mean \pm SEM, (n=9 for all). All data were statistically compared by one-way ANOVA.